THE METABOLIC FATE OF ARACHIDONIC ACID APPLIED TO POTATO TUBER SLICES

S.F. OSMAN and M.J. KURANTZ

*Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118 (U.S.A.)**

(Received February 15th, 1984)
(Revision received July 19th, 1984)
(Accepted July 24th, 1984)

The metabolism of arachidonic acid (AA) and other selected fatty acids by potato (*Solanum tuberosum*) tuber slices was followed with the use of radioactive labeled substrates. The predominant metabolites observed were glycerides. Significant quantities of the unmetabolized acids were also recovered. None of the expected AA metabolites such as hydroperoxides or cyclopentenones that are produced by cell-free systems could be detected under the incubation conditions. The elicitor activity of the fatty acids could not be correlated with their rate of metabolism.

Key words: potato phytoalexins; elicitors; arachidonic acid

Introduction

Hypersensitivity and phytoalexin accumulation result from the interaction of an incompatible fungal race with host plant tissue. The incompatible interaction of *Phytophthora infestans* with potato (*Solanum tuberosum*) tuber slices results in the accumulation of the norsesquiterpene phytoalexin, rishitin, as well as a number of sesquiterpenes of lesser antifungal activity (lubimin, phytuberin and rishitinol to mention a few) [1]. Arachidonic and eicosapentaenoic acids, compounds that elicit the accumulation of rishitin and lubimin, have been isolated from homogenates of *P. infestans* [2]. The methyl ester of AA but not arachidonyl alcohol elicited phytoalexins and the elicitor activity of AA was enhanced by fungal cell wall carbohydrate [3]. The specific activity of AA in the various fungal lipid classes (triglycerides, diglycerides, polar lipids and free acids) is not constant, suggesting that other fatty acids in these fractions may inhibit arachidonic acid activity [4]. In contrast to the polysaccharide and glycoprotein elicitors that have been isolated from other microorganisms [5] it is reasonable to suspect that the active elicitor, in this case, is a metabolite of arachidonic acid, e.g. prostaglandin-like compounds that have been produced by the incubation of unsaturated fatty acids with plant extracts [6]. The ability of plants to synthesize prostaglandin-like molecules from unsaturated fatty acids has been demonstrated by Zimmerman [6]. We wished to determine if similar compounds or other AA metabolites are formed in the interaction of AA and potato tuber slices, and whether such metabolites, if they exist, have elicitor activity. We, therefore, undertook a study of the fate of radioactive AA in potato slices. To our knowledge, there is no report of the metabolism of exogenously added fatty acids by potato tuber slices.

---

*Agricultural Research Service, U.S. Department of Agriculture.

Abbreviations: AA, arachidonic acid; GS/MS, gas chromatography/mass spectrometry; TLC, thin-layer chromatography.

Experimental

Materials and methods

Uniformly labeled ^{14}C fatty acids were purchased from New England Nuclear Corporation* (Boston, MA). Unlabeled fatty acids were acquired from Sigma Chemical Company (St. Louis, MO). Methyl arachidonate was prepared from AA and BF_3-methanol. The purity of all compounds was determined by thin-layer chromatography (TLC) and gas chromatography/mass spectrometry (GS/MS) of the methyl esters. Certified Katahdin seed tubers were obtained from the Agway Corporation (Presque Isle, ME).

GC/MS analysis was carried out on a Hewlett-Packard 5995 instrument fitted with a 12.5 m OV-1 fused silica column. For TLC, Silica gel G 250 or 500-μ plates (Analtech, Newark, DE) were used with the specified solvent systems. Radioactivity on TLC plates was assayed with a Berthold LB 2832 Automatic Linear Analyzer interfaced with an Apple II Computer for data handling. Liquid scintillation counting was carried out on a Beckman LS 8100 Liquid Scintillation Counter.

Incubations

Potato tubers were cut into 2 cm diameter, 0.4 cm thick discs, and rinsed with deionized water. Aliquots of $CHCl_3$ solutions containing the fatty acid or methyl ester were taken to dryness under N_2, and the residue was resuspended in water by sonication for 1 min followed by vortex mixing. Palmitic acid which could not be homogeneously suspended by this technique was converted to the ammonium salt to enhance water solubility. The suspensions (or solutions in the case of palmitic acid) were applied to potato discs at a concentration of 100 μg unlabeled acid/disc (200 μl of suspension) with 2×10^5 cpm/disc of the labeled acid applied. Slices were incubated in Petri dishes wrapped with Parafilm at 18°C, 74% humidity for time intervals up to 24 h.

For verification of metabolites, 100 μg of AA was applied to each of the 30 tuber slices. The same experiment was done with methyl arachidonate. Slices were incubated for 24 h at 18°C.

Isolation

For radioactive experiments, samples were taken at specified intervals and ground in a blendor with $CHCl_3/CH_3OH$ (2:1, v/v) for 1 min, filtered, and the residue re-extracted twice. Combined filtrates were evaporated to dryness and then diluted with $CHCl_3/CH_3OH/H_2O$ as described by Folch et al. [7]. The lipid phase was removed, evaporated to dryness, and made up to volume with $CHCl_3$.

Acyl thioesters were isolated from tuber slices by standard procedures [8,9].

Analysis of radioactivity

Total activity in each fraction was determined by adding aliquots to 10 ml of Aquasol, Universal Liquid Scintillation Cocktail (New England Nuclear). Analysis of the lipid classes was accomplished by TLC fractionation (hexane/diethylether/acetic acid, 60:30:1, v/v/v) with subsequent assaying of the TLC plates for radioactivity with the linear analyzer. Each TLC channel was scanned for 30 min. Standards were used to determine the R_f of the various lipid classes on a separate channel and visualized with phosphomolybdic acid spray reagent.

Verification of metabolites

The lipid fraction from the large scale experiment with unlabeled acids was fractionated by TLC as above. The various zones corresponding to the lipid classes were removed and the gel eluted with 10% CH_3OH in diethyl ether. Extracts were concentrated to dryness under N_2 and saponified and methylated as previously described [10]. Samples were analyzed by GC/MS.

*Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

The polar lipid fraction obtained from potato tuber slices inoculated with a suspension of labeled and unlabeled AA for 30 h at 18°C was applied to a DEAE-cellulose column prepared by the method of Rouser [11] and fractionation and quantitation were carried out according to the procedure described by Galliard [12]. Uninoculated tissue was used as a control.

Determination of elicitor activity

Katahdin tuber discs (2 cm × 0.4 cm) were treated with 0.328 mmol/disc of fatty acids and methyl arachidonate and incubated for 4 days at 18°C. Slices were extracted and analyzed for rishitin as previously described [4].

Results

Labeled arachidonic and linoleic acid, methyl arachidonate and ammonium palmitate were applied to potato tuber slices that were freshly cut. Within 5 h after application the amount of extractable acid (free and esterfied) had diminished dramatically in each case (Fig. 1); further loss, after 5 h, occurred only with the AA. The change in the amount of total recoverable radioactivity with time (Fig. 2) closely paralleled that observed for

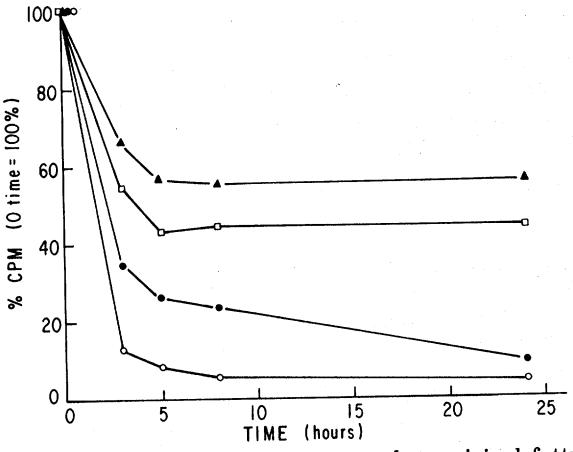

Fig. 1. Percent of label recovered as original fatty acid as a function of time. ▲, palmitic acid; ○, linoleic acid; ●, AA; □, methyl arachidonate.

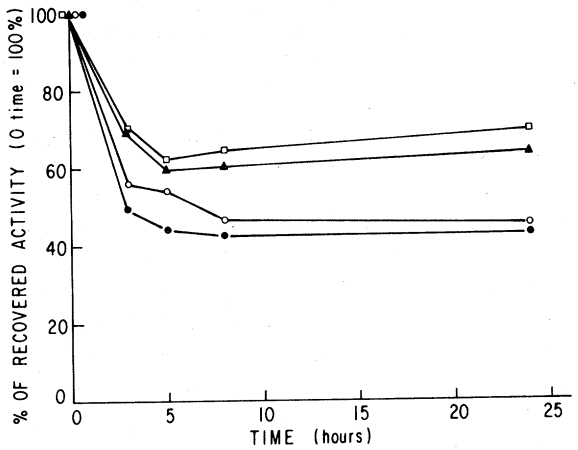

Fig. 2. Percent total recovered radioactivity in lipid fraction as a function of time. ▲, palmitic acid; linoleic acid; ●, AA; □, methyl arachidonate.

fatty acid recovery; however, the level of activity was higher in each instance. Analysis of the extractable lipids which included the polar lipids, diglycerides, triglycerides and free fatty acids (or methyl ester, in the case of methyl arachidonate) (Table I) accounted for all recoverable radioactive metabolites of palmitic and AA and methyl palmitate; linoleic acid metabolism produced a number of additional metabolites that were not identified. AA and linoleic acid were incorporated predominantly into di- and triglycerides, methyl arachidonate was incorporated into glycerides to a lesser extent, and ammonium palmitate was recovered almost exclusively as palmitic acid (the ammonium salt was used for application because the free acid could not be dispersed in water).

The lipid fractions from potato slices treated with AA and its methyl ester were purified by TLC. The purified fractions were transmethylated and the resultant methyl esters were analyzed by GC/MS. The amount of methyl arachidonate in each fraction of the two treatments (Table II) was in good agreement with the distribution of radioactivity in these fractions (Table I). Similar analysis could not be used for linoleic acid or ammonium palmitate-treated slices, because the acids are endogenous to the potato.

Table I. Percent radioactivity incorporated into lipid classes after incubation with acid or ester substrate. PL, polar lipids; DG, diglycerides; TG, triglycerides; FA, free acid; ME, methyl ester; U, unidentified.

Cpd supplied	Incubation time (h)	Metabolites						
		% cpm in fraction						% cpm time h / cpm time 0
		PL	DG	TG	FA	ME	U	
AA	0				100			100
	3	14	0.6	15	70	—	—	50
	5	17	0.7	22	60	—	—	44
	8	17	0	27	56	—	—	43
	24	25	0	54	21	—	—	43
Methyl arachidonate	0	—	—	—	—	100	—	100
	3	5	trace*	5	11	79	—	70
	5	7	trace*	9	15	70	—	62
	8	8	—	14	9	69	—	64
	24	14	—	20	2	64	—	68
Linoleic acid	0	—	—	—	100	—	—	100
	3	40	21	21	19	—	0	56
	5	53	4	23	15	—	6	54
	8	48	4	24	13	—	11	46
	24	51	ND[a]	20	10	—	20	45
Palmitic acid (NH_4^+ salt)	0	—	—	—	100	—	—	100
	3	2	trace	2	96	—	—	69
	5	3		2	95	—	—	60
	8	4		4	92	—	—	60
	24	7		4	88	—	—	63

[a]Not detected.

Table II. GC/MS analysis of lipid classes after a 24-h potato tuber slice incubation with methyl arachidonate and AA. Results are expressed as wt% AA found in the various lipid classes. AA was determined as the methyl ester. Isolation, saponification and methylation of fractions were performed as outlined in Experimental section.

Lipid class	Tuber treatment	
	Arachidonic acid % AA	Methyl arachidonate % AA
Polar lipids	26.1	14.9
Diacylglycerides	4.1	2.0
Free fatty acids	34.0	7.9
Triacylglycerides	35.8	23.5
Methyl esters		51.8

Prefractionation of polar lipids on DEAE-cellulose yielded fractions subsequently separated by one dimensional TLC and scanned for radioactivity on the TLC linear analyzer. All radioactive areas of the chromatogram corresponded in R_f to standard polar

Table III. Comparative elicitor activity of fatty acids.

Rishitin μg/g fresh wt. tuber treatment	
117.5 ± 2.0	(0.328 mmol/slice) AA
122.5 ± 6.3	(0.328 mmol/slice) methyl arachidonate
ND[a]	(0.328 mmol/slice) palmitic acid
ND	(0.328 mmol/slice) linoleic acid
ND	(0.328 mmol/slice) arachidonyl CoA[b]

[a]Not detected.
[b]Sigma Chemical Company.

Table IV. Incorporation of [U-^{14}C]AA into acyl-CoAs and lipid classes by potato tuber slices after a 20-h incubation.

Fraction	cpm (%)
Folch lipid[a]	69.4
Acyl-carrier protein	0.61
Residue	7.0
Polar lipids	8.9
Non-lipid[b]	3.0
Acyl thioesters	11.1

[a]Total lipids—polar lipids.
[b]Aqueous phase of $CHCl_3$/MEOH/H_2O (10:5:3) fractionation of pooled non-polar lipid fractions.

lipids found in potato tubers. Phosphatidyl choline accounted for 40% of the total activity in the polar lipid fraction.

The comparative elicitor activity of the four compounds used in this study is given in Table III. The quantity of compound applied to slices in this experiment was equivalent to the amount applied for metabolic studies.

The incorporation into acyl CoA, and acyl-carrier protein and other polar lipid fractions, is shown in Table IV.

Discussion

The mechanism by which AA elicits phytoalexin production in potato tubers is unknown; however, the intermediacy of AA metabolites such as prostaglandins is an attractive hypothesis in light of what is known of AA metabolism in animal systems. A close parallel to the conversion of AA to prostaglandins that occurs in animal systems, has been found to occur in plants [13,14]. The substrate requirements for the fatty acid conversion to prostaglandin-like compounds are: (a) it be capable of forming the 6-hydroperoxide and (b) it be unsaturated in the *n*-3 position. AA meets both of these requirements. A protein extract of potato tuber tissue converted linoleic acid to 12-oxo-*cis*-10,15-phytodienoic acid [15]. Since Bostock [2] has shown that only AA and eicosapentaenoic acid elicit potato phytoalexins, whereas other fatty acids including linoleic and linolenic acid do not elicit, it seemed conceivable that prostaglandin-like compounds may be active metabolites that are responsible for phytoalexin accumulation when AA is applied to potato tuber slices. The results obtained in our experiments with ^{14}C-uniformly labeled AA are not consistent with such a hypothesis. When labeled AA is applied to potato slices, the only significant metabolites that can be discerned within a 24-h period of incubation are glycerides of AA, primarily triglycerides as determined by capillary GC/MS analysis of the fractions containing radioactivity. We were not able to detect any metabolites that had chromatographic behavior similar to the compounds described by Vick and Zimmerman [13–15]. However, incubation of ^{14}C-linoleic acid appeared to produce such metabolites, since zones of radioactivity on TLC plates in the region one would expect to find the hydroperoxide metabolites were observed. It should be noted that it was not possible to recover more than 60% of the radioactivity supplied, even when solvent extraction of the potato slice was done immediately after application of the acid. This low recovery of radioactivity is not uncommon in experiments with potato slices, even in control situations, and may be due to either complete metabolism to CO_2 and H_2O or irreversible binding to starch (for which we have obtained electron microscopic evidence). Similar recoveries were obtained for all the acids used in these experiments irrespective of their elicitor activity. If an unidentified metabolite is responsible for the phytoalexin accumulation observed, it is either rapidly metabolized or unextractable from the tuber tissue. We had expected to find such metabolites based on not only our knowledge of enzymatic transformation of AA, but also on the basis of what is known about non-enzymatic oxidation of AA [16].

The conversion of the acids to the esters through acyl-CoA intermediates is evidenced by the presence of radioactivity in the acyl-CoA fractions isolated from slices treated

with labeled AA. The conversion of CoA esters to glycerides and free acids may account for the slight increase of radioactivity that was recovered in the non-polar lipid fraction between 5 and 24 h.

The metabolism observed, i.e. conversion of fatty acids to glyceride esters, does not correlate with elicitor activity. Methyl arachidonate, which has similar elicitor activity to AA (Table III), is metabolized to a much lesser extent than AA (Table I), whereas linoleic acid, which has no elicitor activity, is converted to glycerides and other metabolic products at a rate equivalent to that observed for the metabolism of AA (Table I). The inability of the potato tissue slices to metabolize ammonium palmitate is most likely related to the fact that the salt or free acid is not transported to the site of metabolism.

In conclusion, AA is incorporated into potato lipids as glyceride esters with its backbone unaltered. That other enzymatic products such as hydroperoxides or cyclic compounds similar to prostaglandins were not detected, may be a function of the method of incubation, i.e. application of the acid to a slice rather than to a cell-free homogenate. In fact, it appears that AA is less susceptible to non-enzymatic oxidation under these conditions than when simply exposed to air. Although we cannot discount the presence of active metabolites that are below our level of detectability, our ability to account for all recoverable radioactivity as AA suggests that AA is the active form of the elicitor of phytoalexin accumulation on potato slices. The recent report by Stelzig [17] that lipoxygenase inhibitors inhibited phytoalexin production of AA-stressed potato slices would seem, in light of our results to be due to an effect other than inhibiting AA oxidation. Whether AA is responsible for potato phytoalexin accumulation in vivo remains to be determined.

References

1 A. Stoessl, J.B. Strothers and E.W.B. Ward, Phytochemistry, 15 (1976) 855.
2 R.M. Bostock, J.A. Kuc and R.A. Laine, Science, 212 (1981) 67.
3 R.M. Bostock, R.A. Laine and J.A. Kuc, Plant Physiol., 70 (1982) 1417.
4 M.J. Kurantz and S.F. Osman, Physiol. Plant Pathol., 22 (1983) 363.
5 C.A. West, Naturwissenschaften, 68 (1981) 447.
6 D.C. Zimmerman and P. Feng, Lipids, 13 (1978) 313.
7 J. Folch, M. Lees and G.H. Sloane-Stanley, J. Biol. Chem., 226 (1957) 497.
8 M. Mancha, G.B. Stokes and P.K. Stumpf, Anal. Biochem., 68 (1975) 606.
9 J. Sanchez and M. Mancha, Phytochemistry, 19 (1980) 817.
10 H.T. Slover and E. Lanza, J. Am. Oil Chem. Soc., 56 (1979) 933.
11 G. Rouser, C. Galli, E. Lieber, M.L. Blank and O.S. Privett, J. Am. Oil Chem. Soc., 41 (1964) 836.
12 T. Galliard, Phytochemistry, 7 (1968) 1907.
13 B.A. Vick and D.C. Zimmerman, Plant Physiol., 63 (1979) 490.
14 B.A. Vick and D.C. Zimmerman, Plant Physiol., 67 (1981) 92.
15 B.A. Vick and D.C. Zimmerman, Plant Physiol., 64 (1979) 203.
16 M.G. Simic and M. Karel, Autoxidation in Food and Biological Systems, Plenum Press, New York, 1980.
17 D.A. Stelzig, R.A. Allen and S.K. Bhatia, Plant Physiol., 72 (1983) 746.